REMARKS

Status of the Claims

Claims 13-15, 18-20, 33-34, and 37 are pending in the present application. Claims 1-12, 16-17, 21-32, and 35-36 were previously canceled. In view of the Request for Continued Examination (RCE) submitted herewith, Applicant respectfully requests entry of the July 11, 2011, amendment and the instant reply, which is further in response to the March 10, 2011, Final Office Action, and the Advisory Action of August 4, 2011. Reconsideration of the present application is respectfully requested.

Issues under 35 U.S.C. § 103

The Advisory Action, which issued on August 4, 2011, states that the claims submitted on July 11, 2011, will not be entered because the Examiner's new search revealed that U.S. Publication No. 2002/0037584 to Lambiase, as well as PCT Publication No. WO 98/48002, teach that topical administration of NGF can prevent or delay the death of retinal ganglion cells. The Examiner references the background sections of U.S. Publication No. 2002/0037584 and PCT Publication No. WO 98/48002 in support of this allegation. The Examiner further states that the express teaching of the above-cited documents would necessitate further consideration of the proposed claim amendments and likely necessitate a rejection under 35 U.S.C. § 103(a) over at least JP 10-218787 to Okamoto ("Okamoto") in combination with U.S. Publication No. 2002/0037584 or PCT Publication No. WO 98/48002.

The Examiner cites paragraph [0020] of U.S. Publication No. 2002/0037584 and page 7, first paragraph, of WO 98/48002 to support her allegation that these documents describe the topical administration of NGF to prevent or delay the death of retinal ganglion cells. For the reasons set forth below, Applicant disagrees with the Examiner's allegations.

The cited references do not teach or suggest the elements of the pending claims

Applicant notes that the portion of the documents to which the Examiner refers states:

> In particular, some studies have been carried out on animals in order to ascertain the effect of the topical administration of NGF in the treatment of retinal pathologies, for instance in the treatment of acute retinal ischemy (Siliprandi R. et al., Inv.

> Ophthalmol. Vis. Sci., 34:3232, 1993), in the transection of the optical nerve (Carmignoto G. et al., J. Neurosci., 9:1263, 1989) and in the treatment of retinitis pigmentosa (Lambiase A. e Aloe L., Grafe's Arch. Clin. Exp. Ophthalmol., 234: S96-S100, 1996). The results demonstrated that the topical administration of NGF can prevent, or at least delay, the death of the retinal ganglion cells and of the photoreceptors during the above pathologies.

Applicant submits that the above-described passage does not expressly describe or suggest the *topical administration of NGF over an ocular surface of a subject to provide an effective amount of nerve growth factor to retinal ganglion cells and optic nerve* as described in the pending claims. In context, the above-described passage refers to contacting retinal ganglion cells with NGF by injection. This interpretation is clear from the documents cited in the above referenced passage, which describes the intraocular injection of NGF in cats (Siliprandi *et al.*), the intraocular injection of NGF in rats (Carmignoto *et al.*), and the intraocular and retro-ocular injection of NGF in mice (Lambiase *et al.*), *abstracts enclosed.* Accordingly, the "demonstrated" results to which PCT Publication No. WO 98/48002 and U.S. Publication No. 2002/0037584 refer describe the effect of NGF on retinal ganglion cells by eye injection.

Applicant further submits that Okamoto does not remedy the deficiency of U.S. Publication No. 2002/0037584 or WO 98/48002 since Okamoto also fails to teach or suggest the topical administration of NGF over an ocular surface of a subject to provide an effective amount of nerve growth factor to retinal ganglion cells and optic nerve.

Reconsideration and withdrawal of the rejection is respectfully requested.

Conclusion

In view of the above-described remarks and the July 11, 2011, amendment, Applicant submits the pending claims are in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, PhD, Registration No. 46,046, at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized in this, concurrent, and future replies to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

Dated: AUG 10 2011

Respectfully submitted,

By Linda Parker Reg No 46,044

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Attachments

Articles

Nerve Growth Factor Promotes Functional Recovery of Retinal Ganglion Cells After Ischemia

Renata Siliprandi, Roberto Canella, and Giorgio Carmignoto

Purpose. To investigate the effect of a transient complete ischemia on the function of cat retina and to determine whether nerve growth factor (NGF), which was previously shown to enhance retinal ganglion cell (RGC) survival after optic nerve section in the adult rat, can promote recovery of retinal neurons after the ischemic insult.

Methods. Function of distal and proximal retina was assessed by recording the electroretinogram in response to both homogenous flickering light (FERG) and contrast reversing gratings (PERG), respectively, 30 days after the induction of a 60-minute episode of ischemia. Visual evoked potentials in response to contrast reversing gratings were also recorded to evaluate visual acuity and contrast thresholds. Cell survival after ischemia was assessed in retinal whole-mounts stained with cresyl violet. Cats were intraocularly treated with NGF every other day, 3 times a week, for 30 days. Controls were treated with either phosphate buffered saline or cytochrome c.

Results. After ischemia, the FERG was not significantly affected. On the contrary, the PERG, visual acuity, and contrast thresholds were severely impaired. After NGF treatment, PERG response amplitudes were much less reduced compared to controls, and visual acuity and contrast thresholds were virtually normal. In addition, a larger number of presumed RGCs was present in the NGF-treated retinas compared to the cyt c-treated ones.

Conclusions. The more proximally located retinal neurons, in particular RGCs, are highly vulnerable to ischemia. Intraocular NGF treatment was effective in enhancing the survival and functional recovery of these neurons. This suggests that NGF may represent a novel therapeutic agent for the treatment of ischemic ocular pathologies. Invest Ophthalmol Vis Sci. 1993;34:3232–3245.

The loss of the blood supply to the retina and choroid readily affects retinal function,[1–4] resulting in irreversible cell damage.[5–7] Transient interruption of the blood supply can, however, result in a reversible impairment of retinal function. In cats and rabbits, a complete recovery of the electroretinographic b-wave was observed after a 30-minute episode of complete ischemia, whereas only a partial recovery occurred after an ischemic episode of longer duration.[8] In monkeys, after occlusion of the central artery, a complete recovery of the electroretinogram (ERG) and visual evoked potentials (VEPs) followed 97 minutes of ischemia, whereas irreversible damage was observed after 105 minutes of ischemia.[9] Because transient ischemia is involved in many ocular pathologies, these studies may be of clinical relevance in showing a relationship between the duration of the ischemic insult and the degree of functional recovery of retinal neurons. Most of these studies are, however, limited in that they do not provide information on the effects of ischemia on retinal ganglion cell (RGC) function (but see ref. 10).

In the first part of this study, we investigated the effects of episodes of complete retinal ischemia with different duration on the function of cat RGCs by recording the ERG and VEPs in response to pattern reversals (PERG and PVEPs, respectively). The PERG, generated in the proximal retina, is known to be mainly related to RGC activity.[11–13] Results indicate a higher vulnerability to ischemia of RGCs in contrast to the more distal retinal neurons. Results also indicate that 3 days after 60 minutes of ischemia, RGC responses are absent, with partial recovery within a month.

From Fidia Research Laboratories, Abano Terme, Italy.
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Effect of NGF on the Survival of Rat Retinal Ganglion Cells Following Optic Nerve Section

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The ability of NGF (2.5S subunit) to support the survival of adult rat retinal ganglion cells (RGCs) and optic nerve fibers after intracranial section of the optic nerve was investigated. NGF was injected intraocularly at a dose of 3 μg/injection every 2.3 d from the day of axotomy to analysis. Control animals received cytochrome c injections. The survival of RGCs was analyzed in whole-mounted retinas after either cresyl violet staining or labeling with HRP applied to the proximal stump of the optic nerve. Survival times were 5 and 7 weeks. Diameter distribution and number of myelinated optic nerve fibers were assessed in ultrathin cross sections of the optic nerve.

We found that RGCs surviving axotomy were much more numerous following NGF treatment compared with controls. Large-size cells were, in particular, preserved by NGF treatment. The quantitative ultrastructural studies indicated that the number of myelinated optic nerve fibers at 5 and 7 weeks postaxotomy was significantly greater in the NGF group with respect to the cytochrome c group. In agreement with the results obtained at the level of the RGCs, large-diameter axons were, in particular, preserved. We conclude that NGF injected intraocularly is effective in promoting the survival of RGCs and optic nerve fibers at least for a period as long as 7 weeks after intracranial section of the optic nerve.

Retinal ganglion cells (RGCs) of lower vertebrates respond to the optic nerve section by regenerating the injured axons and reestablishing functional connections (for review, see Grafstein, 1986). RGCs of mammals do not possess similar regenerative capacities (Maffei and Fiorentini, 1981; Perry, 1981; Grafstein and Ingoglia, 1982; Hollander et al., 1984; Misantone et al., 1984), although signs of axonal regrowth have been observed in selected experimental conditions (McConnel and Berry, 1982; Richardson et al., 1982; Barron et al., 1986). The reasons for this limited regeneration potential are poorly understood. Evidence is now emerging that the survival and regeneration of injured neurons in the CNS depend, at least in part, on their interactions with surrounding neuronal and non-neuronal tissues (for review, see Aguayo, 1985). Trophic factors have been demonstrated to play important roles for neuronal survival and general growth capabilities in development (Landmesser and Pilar, 1974; Varon and Adler, 1980), as well as in the adult state (for review, see Varon et al., 1987). The prototype of such neuronotrophic factors, the nerve growth factor (NGF) (Levi-Montalcini and Angeletti, 1968; Greene and Shooter, 1980), has now been found to be active at the level of the CNS, especially on forebrain cholinergic neurons (Hefti et al., 1984; Hefti, 1986; Williams et al., 1986; Kromer, 1987; for review, see Thoenen et al., 1987).

In the present study we investigated the effect of repetitive intraocular injections of NGF on the survival of RGCs after intracranial section of the optic nerve. NGF is shown here to promote the survival of a large number of RGCs for period as long as 7 weeks after axotomy. Similar effects were observed at the level of the optic nerve fibers.

Materials and Methods

Animals. Experiments were performed on adult male Long Evans hooded rats. Animals were housed 2 per cage, in a temperature- and humidity-controlled room (22°C, 50%, respectively) on a 12 hour light–dark cycle and were allowed food and water *ad libitum*.

Optic nerve section. In order to avoid possible damage to the blood supply of the retina, the optic nerve was transected intracranially. Animals were deeply anesthetized with 2.5 ml/kg of a mixture of 2.1 gm chloral hydrate and 0.5 gm sodium pentobarbital in 50 ml solution. A frontal craniotomy was made and a portion of frontal cortex was then aspirated to allow a clear visualization of the right optic nerve. Transection was performed between the optic foramen and the chiasm. The completeness of the transection in each experimental animal was subsequently verified at the light microscopic level in semithin cross sections (0.5–1.0 μm) stained with toluidine blue. Operated animals were divided into NGF and cytochrome c-treated groups. Postoperative survival was 5 and 7 weeks.

Drugs and treatment. The 2.5S subunit of NGF was purified from adult male mouse submandibular glands according to the method of Bocchini and Angeletti (1969) and dissolved in buffered saline. The biological activity of the purified NGF, evaluated utilizing fetal chicken dissociated dorsal root ganglion neurons *in vitro* (Skaper and Varon, 1982), was in the range of 1–2 ng protein/trophic unit. Sterile NGF was injected intraocularly every 2.3 d from the day of optic nerve section to the day of death at a dose of 3 μg/injection in approximately 3 μl solution. Rats were anesthetized with diethyl ether. Control animals received equal amounts of cytochrome c (a protein with similar molecular weight and isoelectric point to NGF). Injections were made by means of a glass pipette, with a 50 μm tip, connected via a polyethylene tube with a 25 μl Hamilton syringe. The tip of the pipette was inserted under microscopic guidance through the dorsal limbus of the eye.

Nissl stain. Animals were given a lethal dose of Nembutal and perfused intracardially with normal saline, followed by 10% formolsaline. Retinas were dissected, whole-mounted, stained with cresyl violet as described by Perry and Cowey (1979), dehydrated, and coverslipped. In total, 16 animals were examined. Nine animals (6 NGF-treated and 3 cytochrome c-treated) were analyzed 5 weeks after optic nerve section. Two animals per group were analyzed at 7 weeks. The retinas of 3 adult animals with intact optic nerves were also examined.

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Graefes Arch Clin Exp Ophthalmol. **1996** Aug;234 Suppl 1:S96-100.

Nerve growth factor delays retinal degeneration in C3H mice.

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Abstract

BACKGROUND: The aim of the present study was to investigate the biological role of nerve growth factor (NGF) retinal degeneration in the C3H mouse strain. This strain is characterized by a single gene mutation (rd) which le photoreceptor degeneration resembling human retinitis pigmentosa.

METHODS: Neural retinas from 1- to 25 day-old C3H mice were dissected from outer ocular tissues, dissociated suspension, stained with a vital dye and counted in a hemocytometer. For in vivo study, NGF was injected into th intraocular or retro-ocular area, and at the end of the treatment the mice were killed. The eyes were enucleated, f cut by cryostat into 14-microns serial sections. The serial sections were stained with hematoxylin-eosin and the o nuclear layer (ONL) was measured using a computerized image analysis system.

RESULTS: An intraocular injection of NGF, or repeated retro-ocular injections, induced a significant increase in O thickness compared to controls.

CONCLUSION: Our data show that NGF inhibits retinal degeneration in C3H mice. The mechanism(s) underlyin protective action of NGF against retinal cell death remains to be established.

PMID:8871157[PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

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